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Morphological analysis of leaf growth of maize: responses to temperature and light intensity

H.J. BOS[1], H. TIJANI-ENIOLA[1,2] & P.C. STRUIK[1,*]

[1] C.T. de Wit Graduate School for Production Ecology (PE), Department of Plant Sciences, Crop and Weed Ecology Group, Wageningen University, Haarweg 333, NL-6709 RZ Wageningen, The Netherlands

[2] Present address: Department of Agronomy, University of Ibadan, Ibadan, Nigeria

[*] Corresponding author (fax: +31-317-485572; e-mail: paul.struik@cwe.dpw.wag-ur.nl)

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Abstract

Existing models of leaf-area expansion of Gramineae species are empirical and species-specific. To increase understanding of the mechanisms involved in leaf-area expansion, effects of environmental factors on leaf growth of the non-tillering species maize (*Zea mays* L.) were analysed quantitatively. A growth chamber experiment was carried out with the cultivar Luna including different combinations of temperature (day/night temperatures 13/8, 18/13, 23/18 and 28/23 °C) and photosynthetic-photon-flux density (PPFD) (104, 185 and 277 μmol m^{-2} s^{-1}). At 13/8 °C, a large proportion of the plants died due to prolonged exposure to cold stress. Both high temperatures and high PPFDs increased leaf-appearance rate. Maximum leaf width was highest at intermediate temperatures and high PPFDs, and was strongly related to specific-leaf weight (R^2_{adj} = 0.88). Leaf-elongation rate increased and leaf-elongation duration decreased with temperature, the resultant being a maximum final leaf length at 23/18 °C. Leaf length decreased slightly with PPFD, caused by a shorter leaf-elongation duration. Leaf shape has been described with a new function and was different for Leaves 1 and 2 than for higher-positioned leaves. Leaf width was closely associated with specific leaf weight. The observed relationships can be used in dynamic simulation of leaf area based on plant morphology.

Keywords: Zea mays, leaf area, leaf-appearance rate, leaf-shape model, leaf width, temperature, photosynthetic-photon-flux density

Introduction

Existing models of leaf-area expansion of Gramineae species are empirical and species-specific. To increase understanding of the mechanisms involved in leaf-area expansion, we carried out a research programme in which effects of environmental factors on leaf growth of tillering and non-tillering species were analysed quantitatively. As a tillering species we selected wheat (*Triticum aestivum* L.) and as a non-tillering species we selected maize (*Zea mays* L.). The information from this re-

search will be used to produce a new generic model.

In previous reports (Bos & Neuteboom, 1998a,b), effects of temperature and photosynthetic-photon-flux density (PPFD) on the morphological components of leaf-area dynamics of wheat were described. It was shown that temperature and PPFD mainly affected the rate of increase in number of leaves and hardly the size of the leaves. Number of leaves was largely determined by tiller formation. When a plant lacks this tillering response due to environmental conditions, growth of the main stem fully determines plant adaptation to environmental conditions. A study into the effects of environmental factors on the morphological development of such a plant type could lead to a better understanding of mechanisms involved in the increase of leaf area. Modern maize hybrids only rarely form tillers and are therefore suitable for such a study.

In Gramineae species, visible leaf parts are full-grown, because cell division and elongation take place within the sheath bundle (Dale, 1988). Therefore, the width of leaf parts does not change after emergence of that part. Increase in leaf area of a maize plant can thus be divided into five morphological components: (i) leaf-appearance rate, (ii) leaf-elongation rate (LER), (iii) leaf-elongation duration (LED), (iv) maximum leaf width, and (v) leaf-shape parameters. For growing leaves, the exact shape of the full-grown leaf is needed to calculate the light-exposed leaf area as a function of the fraction of the length that has appeared (Sanderson *et al.*, 1981).

In maize, the effect of temperature on leaf-appearance rate has been studied extensively (Tollenaar *et al.*, 1979; Thiagarajah & Hunt, 1982; Warrington & Kanemasu, 1983). Studies on the effects of leaf position and environmental factors on the four other components are, however, relatively scarce. Therefore, simulation models of growth in leaf area of maize plants are descriptive (Keating & Wafula, 1992; Stewart & Dwyer, 1994). In the current research, the effects of leaf position, temperature and PPFD on the five morphological components are quantified with the objective to improve future modelling efforts on leaf-area expansion of maize plants and to arrive at a more general morphological model for growth of Gramineae plants.

Materials and methods

To avoid variability of environmental factors in time, experiments were done in growth chambers. Maize plants (silage maize hybrid 'Luna') were grown in four growth chambers, each with a different temperature regime. Within a growth chamber, three compartments with different PPFDs were created. Treatments started one day after plant emergence. Plant density was kept low throughout the experimental period.

Plant material and growing conditions

Maize seeds were sown 3 cm deep in 5 L pots filled with a mixture of 33% sandy soil and 67% quartz sand. Three seeds were sown per pot. A total of 540 pots was placed on trolleys in four growth chambers (daily photoperiod 7.00–21.00 h; relative

humidity 70%; temperature 23 °C from 9.00–21.00 h and 18 °C from 21.00–9.00 h).

One day after 50% emergence, plant number per pot and pot number were reduced to obtain a homogeneous population of plants (one plant per pot; 90 pots per growth chamber). Using white curtains, the growth chambers were divided into three compartments (3.20*1.50 m) for the PPFD treatments. Pots were distributed over the three compartments. Each compartment contained 30 plants, resulting in an initial plant density of 19 m^{-2}. Photoperiod and relative humidity remained the same as in the pre-emergence period.

During growth, trolleys were rotated within a compartment approximately every 0.75 ligule-appearance interval (this is the period between the visible appearance of two consecutive ligules) to minimize plant-to-plant variation within treatments. Pots were watered at least once a day. Nutrient solution was supplied every two ligule-appearance intervals based on the expected growth rate and desired high nutrient concentration in the plant material (Scholte, 1987). Trolleys were lowered during growth to obtain a constant PPFD at the top of the plants. The experiment ended at ligule appearance of the seventh leaf.

Plants remained free from pests, diseases and disorders throughout the experiment.

Treatments

Different PPFD treatments were established per compartment. Every compartment ceiling contained six metal halide (Philips HPI 400 W) and six high pressure sodium (Philips AGROSON-T 400 W) lamps. At nine points per compartment, PPFD was measured just above the pots. For the highest PPFD (277 μmol m^{-2} s^{-1}) all lamps were switched on, for the middle PPFD eight lamps (185 μmol m^{-2} s^{-1}), and for the lowest PPFD four lamps (104 μmol m^{-2} s^{-1}). The metal halide lamp/high pressure sodium lamp ratio was 1:1 for every treatment.

Temperature treatments (day/night: 13/8, 18/13, 23/18 and 28/23 °C) were set per growth chamber, based on the conditions in the 185 μmol m^{-2} s^{-1} compartments. On average, during the PPFD period, air temperature was 0.5 °C higher at 277 μmol m^{-2} s^{-1} and 0.5 °C lower at 104 μmol m^{-2} s^{-1} compared to the air temperature at 185 μmol m^{-2} s^{-1}. Day temperatures were set for the period 9.00 – 21.00 h, night temperature occurred from 21.00 – 9.00 h. The growth cabinets required less than half an hour to switch from day to night temperature or *vice versa*.

The range of temperatures established assured large differences in the increase in leaf area.

Measurements

Six plants per treatment were harvested every ligule-appearance interval, starting from ligule appearance of Leaf 3 up to ligule appearance of Leaf 7 (five harvests). After every harvest, the remaining pots were rearranged to minimize inter-plant competition (plant density at last harvest 3.9 m^{-2}). At each harvest, length and maximum width of all leaves were measured with a ruler. For one plant, leaf width of full-

grown leaves was measured at six or seven equidistant places covering the whole leaf length to determine leaf-shape parameters. After this, plants were dissected into roots and shoots, and the shoot into separate visible leaves and internodes/sheaths. Dry weight of these parts was measured after drying the material in a forced-air ventilated oven at 70 °C for 16–24 h until constant weight.

On several occasions throughout the experimental period the length (i.e. from the leaf tip to the last visible ligule) of growing leaves (except Leaf 1) was measured with a ruler to determine leaf-elongation rate and leaf-elongation duration, until the leaf was full-grown. These measurements were done approximately four times per ligule-appearance interval during the same period of each day of measuring on six plants which were used for the last harvest.

Definitions and calculations

Leaf positions were counted acropetally. Leaf appearance was defined as the moment the tip of a leaf blade reached above the uppermost visible ligule. Number of growing leaves was defined as the number of visible leaves not yet showing their ligule. Number of appeared leaves was the number of full-grown leaves plus the number of growing leaves. A leaf was full-grown when its ligule was visible.

Leaf-elongation rate (LER) was assumed to be constant until the leaf was full-grown. LER and leaf-elongation duration (LED) were estimated with a two-step regression as was done in Bos & Neuteboom (1998b). Data of recently full-grown leaves in the destructive harvests were used to analyse full-grown leaf length, maximum leaf width, dry weight and specific-leaf weight (SLW). Shape of full-grown leaves was evaluated with the Sanderson model (Sanderson *et al.*, 1981):

$$\frac{w}{W} = \sin^{\alpha}\left(\frac{\pi}{2a}\frac{x}{X}\right) \qquad (1)$$

where w is the leaf width at distance x from the leaf tip, W the maximum leaf width, X the full-grown leaf length, a the ratio of x/X at the position of maximum leaf width and α a constant that allows for differences in leaf shape. Values for a and α are limited:

$$0.5 < a \leq 1$$
$$\alpha > 0$$

because $w/W \geq 0$ and maximum width does not occur at the leaf base for maize leaves. For full-grown leaves, leaf area can be directly derived from leaf length and maximum leaf width:

$$\textit{Full-grown leaf area} = k^{*}\ \textit{Maximum leaf width}\ ^{*}\ \textit{Full-grown leaf length} \qquad (2)$$

The parameter k (a shape factor) was calculated by numerical integration of Equation 1, in which α accounted for differences in leaf shape.

Statistical analysis

It was assumed that differences in plant growth between growth chambers could be completely attributed to temperature, because the growth chambers were of the same type, conditions could be controlled well and differences in temperature between growth chambers were large. Although we realize that individual plants are actually subsamples, we feel that it is justified to use each plant as a replicate. An analysis of variance was carried out to investigate the significance of treatment effects and to calculate the least-significant difference (LSD) ($P = 0.05$). Effects of number of appeared leaves, temperature and PPFD on leaf area per plant were estimated with a stepwise-regression method (Montgomery & Peck, 1982). Linear, quadratic and interaction terms of the quantitative variables being number of appeared leaves (N_{LF}), temperature (T) and PPFD (L) were used as independent variables. The analysis started with the fit of an empty model. One by one terms were added to improve R^2_{adj}, until six terms were added.

Results

Due to continuous exposure to cold stress, a large portion of the plants grown at 13/8 °C died in an early stage, especially at 104 μmol m^{-2} s^{-1}. We therefore decided to stop the 13/8 °C treatment before seven leaves were full-grown. Data of the 13/8 °C treatments are shown in the graphs, but have not been included in the statistical analyses and calculation of main effects of PPFD. In the other growth chambers, at higher temperatures, death of leaves was negligible.

Leaf area and number of leaves per plant

The leaf area per plant increased almost exponentially with time (Figure 1, Quadrant I). Relative growth rate of leaf area (RGR_{LA}= the slope of ln(leaf area) *vs.* days after emergence (DAE)) significantly increased with temperature. Differences between 23/18 and 28/23 °C were only significant at the highest PPFD (Table 1). Photosynthetic-photon-flux density affected RGR_{LA} significantly but less than temperature did; RGR_{LA} was highest at 185 μmol m^{-2} s^{-1}. The lower RGR_{LA} at 277 μmol m^{-2} s^{-1} compared to 185 μmol m^{-2} s^{-1} was caused by a lower RGR_{LA} in the last harvest interval.

Number of leaves increased linearly with time up to Harvest 4 (Figure 1, Quadrant III). In Harvest 5, tassels had appeared for most treatments and this harvest was therefore excluded from calculations of leaf-appearance rate. Leaf-appearance rate (i.e. slope of number of leaves *vs.* DAE) significantly increased with temperature, although less pronounced above 23/18 °C (Table 2). To a lesser extent than observed for temperature, leaf-appearance rate also increased with an increase in PPFD.

The number of appeared leaves was strongly related to ln(leaf area per plant) (Figure 1, Quadrant II). The stepwise regression showed that for temperatures above 13/8 °C a second order polynomial of number of appeared leaves accounted for

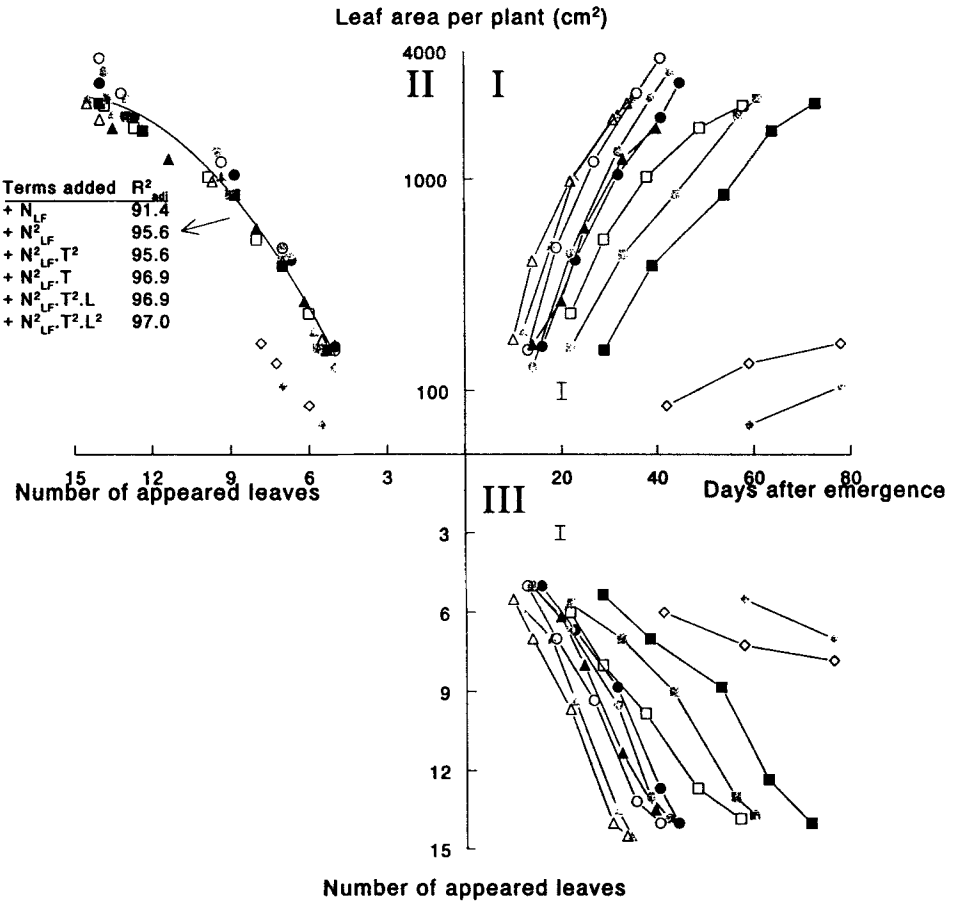

Figure 1. Leaf area per plant in relation to days after emergence and number of appeared leaves (i.e. all visible leaves including the ones that were still growing). Quadrant I: increase of leaf area per plant (log scale) with days after emergence.The bar indicates average LSD (P=0.05). Quadrant II: relation between leaf area per plant (log scale) and number of appeared leaves. The result of the stepwise regression (see text) with independent variables number of appeared leaves (N_{LF}), temperature (T) and PPFD (L) is given (13/8 °C treatments excluded). The fitted line represents the regression model with only the first two terms (N_{LF} and N^2_{LF}) included. Quadrant III: increase of number of appeared leaves with days after emergence. The bar indicates average LSD (P=0.05). Open symbols: PPFD=277 µmol m^{-2} s^{-1}; symbols in grey: PPFD=185 µmol m^{-2} s^{-1}; black symbols: PPFD=104 µmol m^{-2} s^{-1}. The shape of the symbols indicates the temperature treatment: 13/8 °C: ◇; 18/13 °C: □; 23/18 °C: ○; 28/23 °C: △.

95.6% of the variation in ln(leaf area per plant) (N_{LF} and N^2_{LF} terms, Figure 1, Quadrant II). Effects of temperature and PPFD were larger at higher leaf number per plant (T and L interacted with N_{LF}). At comparable numbers of appeared leaves, leaf area per plant was greater at 23/18 than at 18/13 or 28/23 °C (N^2_{LF}.T and N^2_{LF}.T^2 terms) and plants grown at 104 µmol m^{-2} s^{-1} had a lower leaf area than at 185 or 277 µmol m^{-2} s^{-1}, an effect that increased slightly with an increase in temperature (N^2_{LF}.T^2.L

Table 1. RGR_{LA} (d^{-1}) calculated from Figure 1, Quadrant I by linear regression.

PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	Temperature (°C)			
	13/8	18/13	23/18	28/23
104	–	0.060	0.096	0.095
185	0.022	0.068	0.110	0.108
277	0.019	0.061	0.109	0.101

LSD ($P = 0.05$) = 0.007

and $N^2_{LF}.T^2.L^2$ terms). Using predictions of the full 6-term regression model with N_{LF}=14, leaf area per plant ranged from 17.5 dm^2 for the [28/23 °C, 104 $\mu mol\ m^{-2}\ s^{-1}$] treatment to 32.7 dm^2 for the [23/18 °C, 185 $\mu mol\ m^{-2}\ s^{-1}$] treatment. This shows that treatments had large effects on leaf area per plant at later stages of development.

Area, length and maximum width of full-grown leaves

Figure 2 shows the separate effects of temperature and PPFD on full-grown area, length and maximum width of Leaves 1 to 7. For all treatments, the increase of length with leaf position was sigmoidal, while the maximum width was almost constant for Leaf positions 1 and 2 and increased linearly for higher leaf positions. As a result, the increase of area with leaf position was exponential-linear.

Interactions between effects of temperature and PPFD were not significant for most leaves and are not shown. Leaf area was significantly smaller at 18/13 °C than at 23/18 °C, mainly because leaves were shorter (Figure 2). Area of leaves grown at 28/23 °C was significantly smaller than at 23/18 °C mainly due to a lower maximum leaf width. The effect of PPFD on leaf area depended on leaf position. Leaf area was slightly (but significantly) larger for low PPFDs at Leaf position 2 caused by longer leaves. However, for Leaf positions 4 to 7 the effect reversed, because the negative effect of low PPFD on maximum leaf width became more important than its positive effect on leaf length.

Table 2. Leaf-appearance rate (d^{-1}) calculated from Figure 1, Quadrant III by linear regression (last harvest excluded).

PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	Temperature (°C)			
	13/8	18/13	23/18	28/23
104	–	0.19	0.30	0.34
185	0.08	0.21	0.31	0.39
277	0.05	0.24	0.35	0.40

LSD (P=0.05) = 0.03

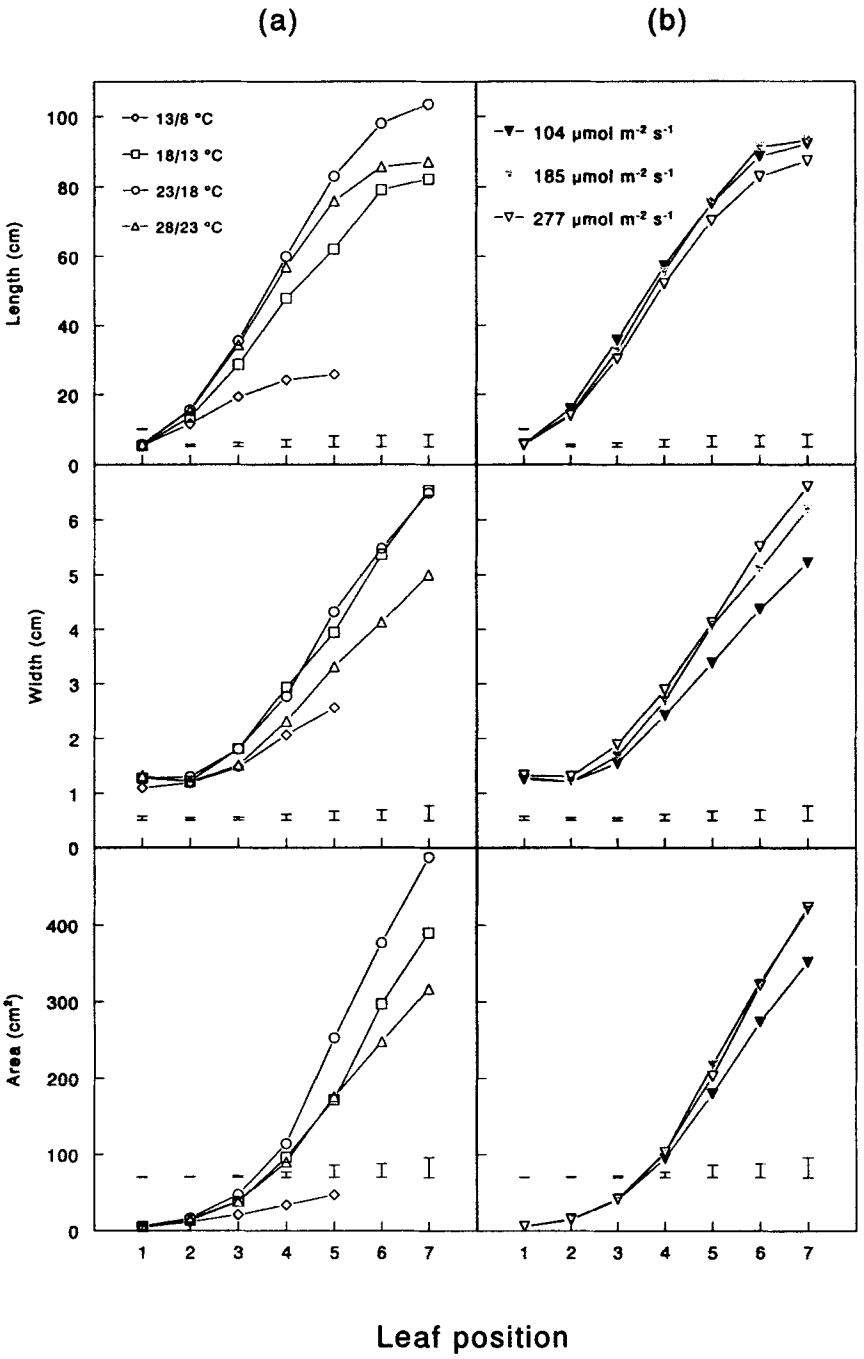

Figure 2. Length, maximum width and area of full-grown Leaves 1–7, (a) averaged per temperature treatment (°C) and (b) averaged per PPFD treatment. Bars indicate the LSD ($P = 0.05$).

Dry weight of leaves and SLW

Just as for leaf area (Figure 2), dry weight per leaf increased expolinearly with leaf position (Figure 3). Since the linear increase of dry weight was larger than that of leaf area, SLW increased from Leaf 3 onwards (Figure 3).

Interactions between temperature and PPFD effects are not shown in the graphs. For Leaves 4, 6 and 7, interactions between effects of temperature and PPFD on SLW were significant, because temperature effects were stronger at low PPFDs. Effects of temperature on dry weight were qualitatively equal to effects on leaf area (23/18 > 18/13 > 28/23 > 13/8 °C). The resultant SLW decreased with temperature above 18/13 °C. More pronounced than for leaf area (Figure 2), dry weight of leaves at 104 μmol m^{-2} s^{-1} was significantly less than at 185 or 277 μmol m^{-2} s^{-1}, the latter two were not significantly different (Figure 3). The resultant SLW increased with PPFD on all leaf positions.

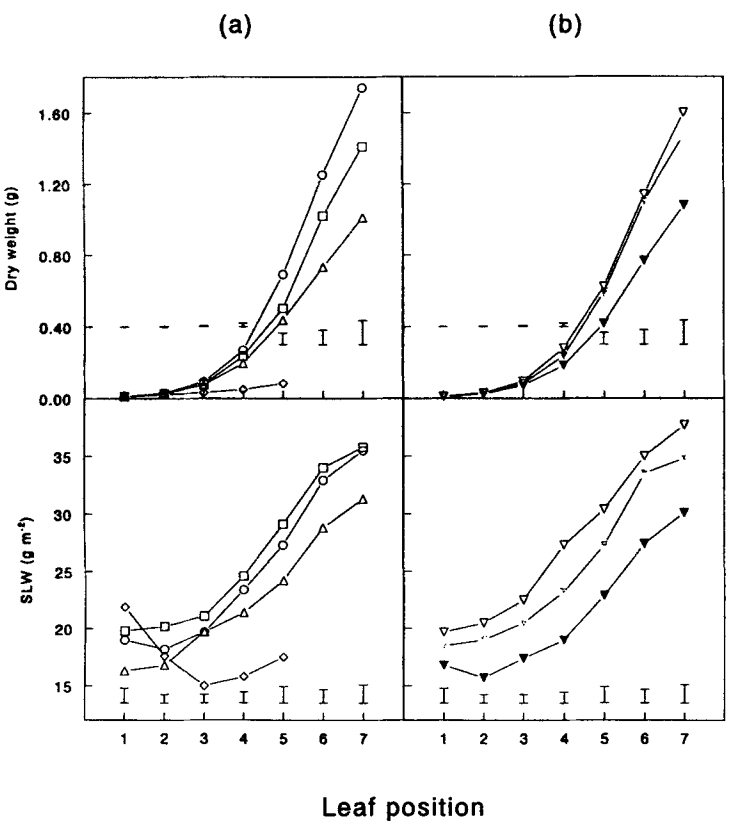

Figure 3. Dry weight and SLW of Leaves 1–7, (a) averaged per temperature treatment and (b) averaged per PPFD treatment. Symbols: 13/8 °C: ◇; 18/13 °C: □; 23/18 °C: ○; 28/23 °C: △; dark (inversed) triangle: 104 μmol m^{-2} s^{-1}; grey triangle 185 μmol m^{-2} s^{-1}; open triangle: 277 μmol m^{-2} s^{-1}. Bars indicate the LSD ($P = 0.05$).

Leaf elongation

Rate of leaf elongation increased with leaf position up to Leaf 5 and then decreased. This increase was more pronounced at high temperatures than at low temperatures (Figure 4). Leaf-elongation duration increased linearly with leaf position.

Interactions between effects of temperature and PPFD on rate and duration of leaf elongation were not significant for most leaves and are not shown. Leaf-elongation rate was significantly greater and LED was significantly shorter for higher temperatures. The shorter Leaves 5 and 6 at 28/23 °C compared with 23/18 °C (Figure 2) were related to the sharp decrease of LER with leaf position at 28/23 °C (Figure 4a). Leaves grown under high PPFDs showed a significantly higher LER compared to leaves grown under low PPFDs for Leaf positions 2 to 5, but differences were small. Duration of leaf elongation was significantly longer at low PPFDs than at high PPFDs for all leaves. Differences between PPFDs for LED were slightly larger than

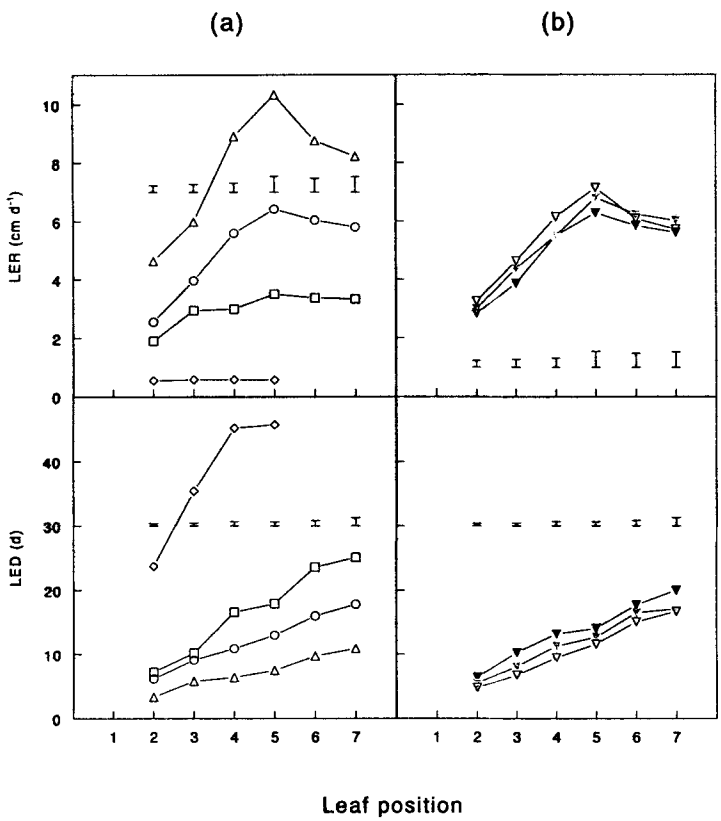

Figure 4. LER and LED of Leaves 2–7, (a) averaged per temperature treatment and (b) averaged per PPFD treatment. Symbols 13/8 °C: ◇; 18/13 °C: □; 23/18 °C: ○; 28/23 °C: △; dark (inversed) triangle: 104 μmol m^{-2} s^{-1}; grey triangle 185 μmol m^{-2} s^{-1}; open triangle: 277 μmol m^{-2} s^{-1}. Bars indicate the LSD ($P = 0.05$).

for LER. Therefore, the fact that leaves under lower PPFDs were longer (Figure 2) was caused by the relatively large positive effect of low PPFD on LED.

Leaf shape

The Sanderson model (Equation 1) did not fit Leaf positions 1 and 2 well, because the maximum width occurred close to the leaf tip. Therefore, another two-parameter model was developed, which accounts for maximum width from leaf base to leaf tip. This model was very similar to the Sanderson model:

$$\frac{w}{W} = \sin^{\alpha}\left(\frac{\pi}{2b^{\beta}}\left(\frac{x}{X}\right)^{\beta}\right) \qquad (3)$$

where b is the ratio of x/X at the position of maximum leaf width and ß a constant that allows for differences in leaf shape. For low values of ß the leaves tend to be wider towards the leaf tip relative to the maximum leaf width. Also for this new model, the values of b and β, are limited:

$$0 > b \leq 1$$

$$0 < \beta < \frac{\ln 0.5}{\ln b}$$

Figure 5 shows an example of a fit of the Sanderson model and the new model for a representative Leaf 2 and 6. The Sanderson model assumes that leaves have an axis of symmetry through the point where maximum leaf width occurs, which is unrealistic especially for lower leaves. The maximum leaf width for Leaf 2 in Figure 5a occurred below $x/X = 0.5$, which is outside the range of the Sanderson model. The maximum width for Leaf 6 occurred above $x/X = 0.5$, and both models gave a similar fit (Figure 5b). Both models were fitted through leaf length and width data of recently full-grown Leaves 1–7 (13/8 °C treatments excluded). The new model accounted for a greater proportion of the variation for Leaves 1 and 2, while for Leaves 3 to 7 the models performed similarly (Figure 6). The parameters b and β, of the new model increased with leaf position for lower leaves, but remained fairly stable from Leaf 4 to 7 (Figure 6). They were not influenced by temperature or PPFD (not shown). The parameter k (Equation 2) decreased from 0.80 for Leaf 1 and 2 to 0.70 for Leaf 4 to 7.

Discussion

Limitations of the current experiment

The current experiment was carried out in growth chambers. PPFD levels were therefore relatively low. However, the data on rate of leaf appearance, leaf growth and dry weight were comparable with those found under field conditions, at least at lower temperatures (Bos *et al.*, 2000). We are therefore confident that the results obtained can be translated to field scale. The inter-plant competition was kept low due to frequent sampling and possible changes in the ratio of red/far-red of the photon

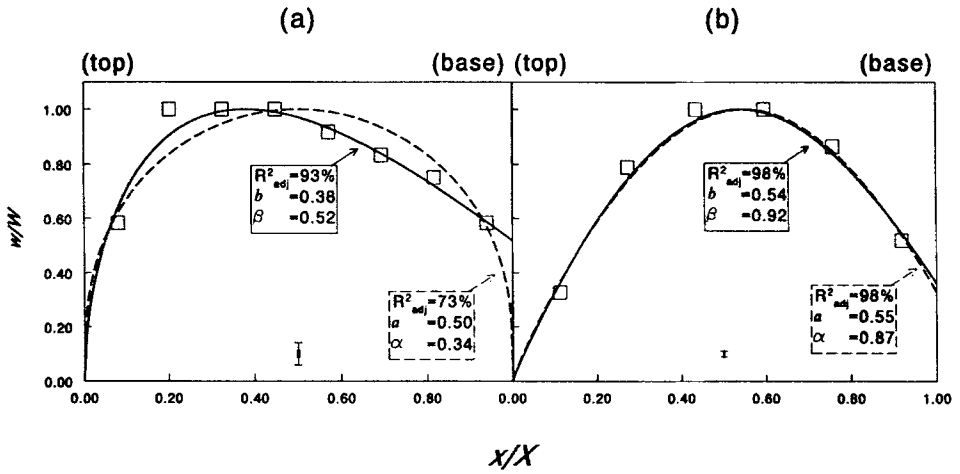

Figure 5. w/W as a function of x/X for a representative Leaf 2 (a) and 6 (b). The dashed line represents the fit of the Sanderson model (Equation 1), the solid line the fit of the new model (Equation 3). R^2_{adj} and parameter estimates are shown in the rectangles. The bars indicate the measurement error of the ruler that was used (+/– 0.5 mm).

flux caused by the continuous adaptation of the plant density during the experiment will not have affected the outcome of the experiment (cf. Bos *et al.*, 2000).

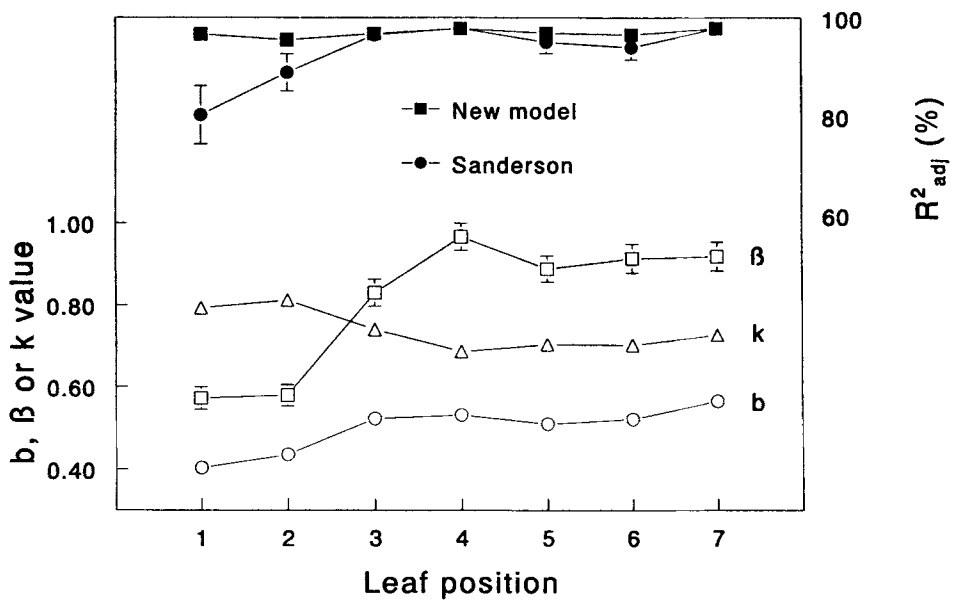

Figure 6. Estimated parameter values of the new model and R^2_{adj} of the two models for Leaf positions 1 to 7. Data points are averages of the 9 treatment combinations (13/8 °C was excluded), with 1 plant per treatment. Bars indicate twice the standard error of the mean. Bars smaller than the marker size do not appear.

Dynamics of leaf number

Leaf-appearance rate was faster at higher temperatures and to a lesser extent at higher PPFDs. The current data set has been compared with growth chamber data from Tollenaar *et al.* (1979) (PPFD = 500 μmol m^{-2} s^{-1}; daylength = 15 h d^{-1}; six hybrids, all singles crosses from North American inbred lines) and Thiagarajah & Hunt (1982) (PPFD = 620 μmol m^{-2} s^{-1}; daylength = 15 h d^{-1}; hybrid A498 x CG10) (Figure 7). Generally, leaf-appearance rates were similar, but at 28/23 °C they were somewhat lower for the current data set. This difference could be due to the lower PPFDs of the current experiment compared to the other experiments, or to cultivar effects, which can, especially at higher temperatures, be strong (Ellis *et al.*, 1992).

Positive effects of PPFD on leaf-appearance rate of maize have been found by Gmelig Meyling (1973) and Struik (1983) and could be due to a slight increase of temperature at the growing point with PPFD.

Individual leaf growth

In the current research, area growth of individual leaves has been separated into maximum leaf width, LER and LED. Temperature and PPFD affected these three components in different ways, which confirms earlier findings with small cereals and grasses (Friend *et al.*, 1962; Allard *et al.*, 1991) and maize (Hesketh & Warrington, 1989).

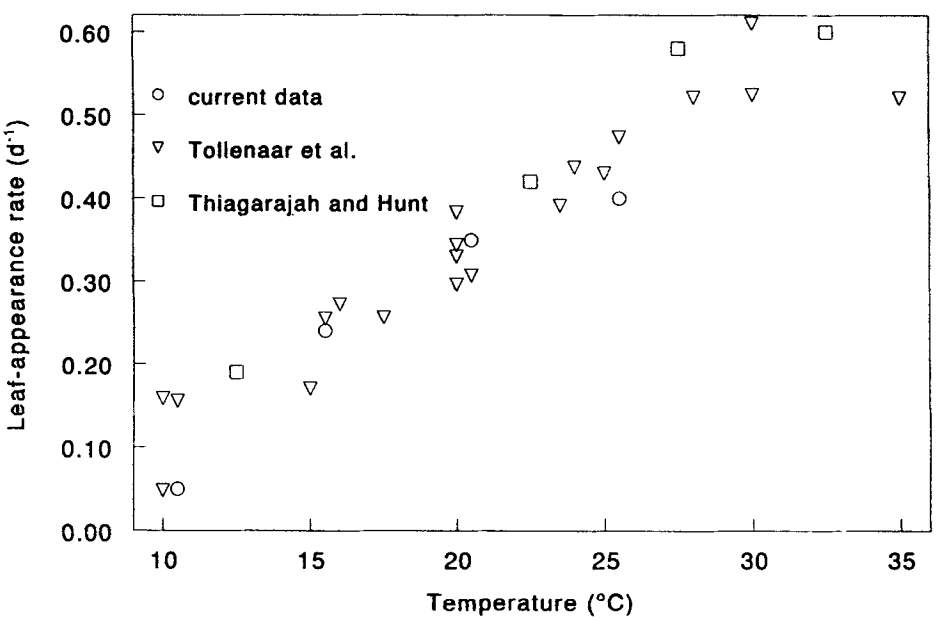

Figure 7. Leaf-appearance rate at different temperatures for the current data set at 277 μmol m^{-2} s^{-1}, data from Tollenaar *et al.* (1979) and Thiagarajah & Hunt (1982).

The width of leaves has received little attention in literature and it is unknown how the width is related to other plant parameters. However, for a mechanistic model it is necessary to find simple relationships with physiological background. In the current research, effects of leaf position, temperature and PPFD on leaf width (Figure 2) were similar to effects on SLW (Figure 3) and a good correlation between the two existed (Figure 8). A possible physiological explanation for this relation is that not only SLW is determined by carbohydrate availability (Thiagarajah & Hunt, 1982; Van Loo, 1993; Grant & Hesketh, 1992), but also maximum leaf width. Leaf width is well related to the number of cell rows across the width (Borrill, 1961; Forde, 1966; Jewiss, 1966) and accordingly to the basal circumference of the shoot apex when the primordium is initiated (Abbe *et al.*, 1941; Robson *et al.*, 1988). The size of the shoot apex is related to the growth rate of the shoot (Pieters, 1986), and thus to carbohydrate availability.

Leaf-elongation rate increased with leaf position up to a maximum, after which it remained stable or declined (Figure 3). This was also found for reproductive barley plants (Kirby, 1973), vegetative perennial ryegrass (Robson, 1973), tall fescue (Skinner & Nelson, 1994) and maize (Grant & Hesketh, 1992) plants. For wheat plants (Bos & Neuteboom, 1998b), effects of PPFD on LER were small. This is in agreement with studies on temperate species (Kemp & Blacklow, 1980; Kemp, 1981; Sambo, 1983), which showed that LER depends only on carbohydrate supply at very low levels of carbohydrates. The longer leaves formed at lower PPFDs were related to a longer LED, which was also found for wheat (Bos & Neuteboom, 1998b) and tall fescue (Allard *et al.*, 1991).

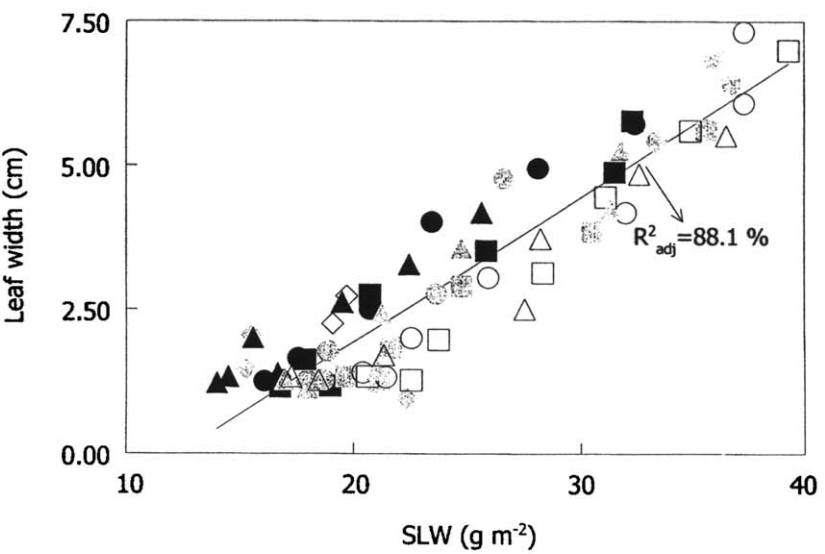

Figure 8. Relation between maximum width and SLW of Leaves 1 to 7. Every data point represents the average value per leaf position per treatment. Symbols: Open symbols: PPFD=277 µmol m^{-2} s^{-1}; symbols in grey: PPFD=185 µmol m^{-2} s^{-1}; black symbols: PPFD=104 µmol m^{-2} s^{-1}. The shape of the symbols indicates the temperature treatment: 13/8 °C: ◇; 18/13 °C: □; 23/18 °C: ○; 28/23 °C: △.

For grasses and small cereals the development of successive leaves is interlocked (Skinner & Nelson, 1995; Tesařová *et al.*, 1992). In these species the number of growing leaves on one stem remains constant. However, in the current study with maize, leaf-appearance rate remained rather constant (Figure 1), while the LED increased with leaf position (Figure 3). Figure 9 shows that as a result of both, the number of growing leaves increased with number of full-grown leaves, especially between 5 and 6 full-grown leaves. Temperature did not significantly affect this relation, which confirms earlier findings of Thiagarajah & Hunt (1982) and Hesketh & Warrington (1989). Low PPFDs significantly decreased the number of growing leaves as a function of number of full-grown leaves (Figure 9). Apparently, as maize plants develop, the number of growing leaves on one stem increases, while for grasses and small cereals the number of growing leaves increases by the formation of tillers.

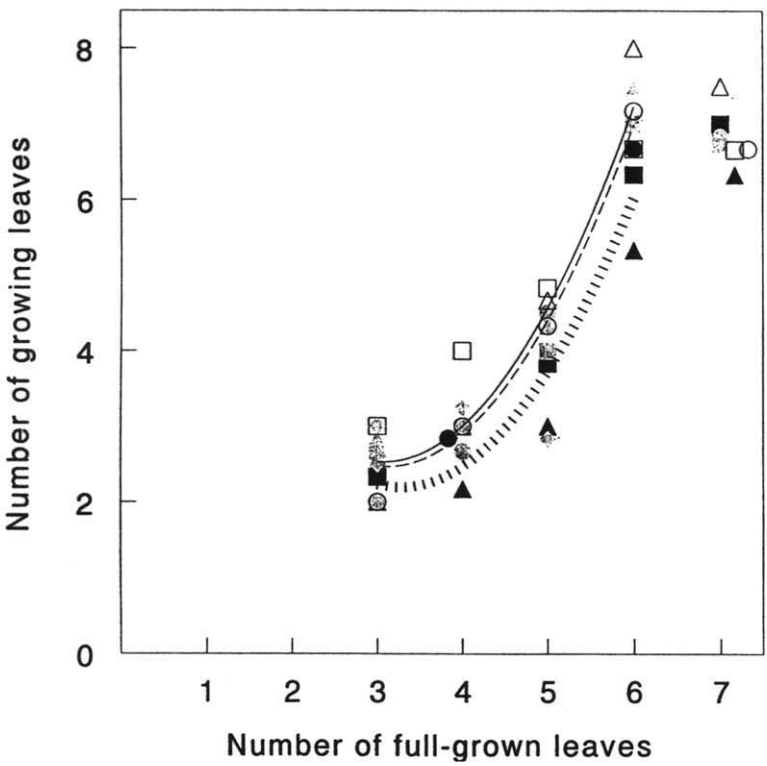

Figure 9. Number of growing leaves in relation to number of full-grown leaves. Every data point represents the average value per harvest per treatment. Symbols: Open symbols: PPFD=277 µmol m^{-2} s^{-1}; symbols in grey: PPFD=185 µmol m^{-2} s^{-1}; black symbols: PPFD=104 µmol m^{-2} s^{-1}. The shape of the symbols indicates the temperature treatment: 13/8 °C: ◇; 18/13 °C: □; 23/18 °C: ○; 28/23 °C: △. The lines are the result of a forward stepwise regression for the first four harvests using the interactive linear and quadratic terms of number of full-grown leaves, temperature and PPFD as independent variables. The stepwise regression was halted after no significant ($P > 0.05$) term could be added. Short dashed line: 104 µmol m^{-2} s^{-1}; long dashed line: 185 µmol m^{-2} s^{-1}; solid line: 277 µmol m^{-2} s^{-1}.

Leaf shape

The new two parameter model (Equation 3) described leaf shape of Leaf positions 1 and 2 better than the Sanderson model (Equation 1), because in the new model the maximum width can occur at any point along the whole length of the leaf. Data for higher leaf positions fitted equally well to both models. Sanderson *et al.* (1981) validated their model on higher Leaf positions 6 to 14. The new model appeared to be more flexible in describing the shape of lower positioned leaves and is therefore wider applicable than the Sanderson model.

Leaf positions 1 and 2 showed different values for b and β than higher leaf positions. It is a common finding that the first leaves of a seedling plant have a different shape and internal structure than higher positioned leaves (Eames, 1961).

Towards a dynamic mechanistic model

Both the range of temperatures (13/8–28/23 °C) and PPFDs (104–277 μmol m^{-2} s^{-1}) affected the increase of leaf area per plant in time significantly, as a result of both differences in leaf-appearance rate and, especially for higher leaf positions, leaf size (Figure 1). This finding disagrees with the proposition of Dwyer & Stewart (1986), that under non-drought conditions the full-grown leaf area of maize is only a function of leaf position. In dynamic mechanistic models, leaf-appearance rate, LER, LED and maximum leaf width should be incorporated separately, because temperature and PPFD affect these parameters in a different way. Such models will be more accurate and wider applicable than current models for Gramineae species. At our Department we are in the process of developing such a model.

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Appendix. Abbreviations.

DAE = days after emergence (d)
LED = leaf-elongation duration (d)
LER = leaf-elongation rate (cm d^{-1})
N_{LF} = number of leaves appeared (# $plant^{-1}$)
L = photosynthetic-photon-flux density (μmol m^{-2} s^{-1}), used in formula only
PPFD = photosynthetic-photon-flux density (μmol m^{-2} s^{-1}), used in text and tables
RGR_{LA} = relative growth rate of leaf area (d^{-1})
SLW = specific-leaf weight (g m^{-2})
T = temperature (°C)